MYCOTOXIGENIC FUNGI, MYCOTOXINS, AND MANAGEMENT OF RICE GRAINS

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Mycotoxin contamination in certain agricultural commodities has been a serious concern for human and animal health. Mycotoxins are substances produced mostly as secondary metabolites by filamentous fungi that grow on seeds, grains, and feed in the field, or in storage. The major mycotoxin-producing fungi are species of Aspergillus, Fusarium, *and* Penicillium. *Aflatoxins, fumonisins, trichothecenes, ochratoxins, cyclopiazonic acid, patulin, deoxynivalenol, zearalenone, citrinin, gliotoxin, and sterigmatocystin are some of the important mycotoxins. This paper reviews the mycotoxigenic fungi, their levels of mycotoxins, and their management by using botanicals, microbiologicals, and cooking methods in rice. The data from detailed investigations on rice seeds and grains help to provide safe grains for consumption and export, and prioritize future research programs.*

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Introduction

Rice (*Oryza sativa* L.) is the most important staple food crop in India and the bulk of rice is grown in *kharif* or the wet season. Frequent and heavy rainfall and floods, particularly near harvest, in coastal areas in eastern, southern, and western regions of the country wet the crop and make panicles more prone to invasion by fungi and bacteria. During the wet season, sun drying practiced by most farmers may not adequately reduce the moisture content in grains. Thus, rice grains with moisture content higher than the desired level enter the storage system. As a result, invasion by both field and storage fungi takes place.

The major mycotoxigenic fungi in rice are *Aspergillus* sp. (Reddy et al., 2004), *Fusarium* sp. (Abbas et al., 1999) and *Penicillium* sp. (Makun et al., 2007). The harmful effects of such fungal invasion are glume or grain discoloration, loss in viability and quality and toxin contamination. Aflatoxins (Liu et al., 2006; Mangala et al., 2006), fumonisins (Abbas et al., 1997; Abbas et al., 1999), trichothecenes (Llewellyn et al., 1988), ochratoxin A (Makun et al., 2007; Reddy, Reddy, and Muralidharan, 2007), cyclopiazonic acid (Trung et al., 2001), patulin (Rao et al., 2005), zearalenone, deoxynivalenol (DON) (Megalla et al., 2007), citrinin (Abd-Allah and Ezzat, 2005), gliotoxin (Richard et al., 1989), and sterigmatocystin (Sugimoto et al., 1977) are the important mycotoxins reported in rice. Mycotoxin contamination is less commonly reported for rice than for many other cereal crops (Tanaka et al., 2007), but rice represents a very good substrate for fungal growth and toxinogenesis since it is used as an ideal culture medium to test the toxigenic potential of fungal isolates (Bars and Bars, 1992). Among these rice mycotoxins, aflatoxin B_1, (AFB1), fumonisin B_1 and ochratoxin A are the most toxic for mammals and have hepatotoxic, teratogenic, and mutagenic activity, causing damage such as toxic hepatitis, hemorrhage, edema, immunosuppression, hepatic carcinoma, equine leukoencephalomalacia, esophageal cancer, and nephrotoxicity (Norred, 1993; Santos et al., 2001; Altuntas et al., 2003). AFB1 has been classified as a group 1 human carcinogen and fumonisin B_1 and B_2 as group 2B carcinogens by the International Agency for Research on Cancer (1993). Several mycotoxicoses in humans and animals have been reported due to the consumption of

mycotoxin-contaminated food and feed (Peraica and Domijan, 2001; Reddy and Raghavender, 2007).

Mycotoxin production is unavoidable and at times unpredictable, which makes it a unique challenge to food safety. Decontamination of mycotoxin-contaminated food is not fully successful, and control of mycotoxins is the need of the hour. The development of integrated management strategies is therefore essential to ensure food safety. Whereas the complete elimination of mycotoxins from food commodities seems an impossible goal, it is important to ensure that their levels should not threaten human health. Though prevention is the best control strategy, mycotoxin contamination will still sometimes occur. However, postharvest control and decontamination procedures represent important tools in avoiding exposure to mycotoxins. Several decontamination strategies have been reported for various mycotoxins in a wide array of agricultural commodities, and specific information on each method is accessible in the literature. This paper will review mycotoxigenic fungi, mycotoxins, and efficacy of botanicals, microbiologicals, agrochemicals, and physical decontamination strategies for mitigating risks associated with exposure to mycotoxin-contaminated rice.

Mycotoxigenic Fungi in Rice Grains

Mycotoxin contamination often occurs in the field prior to harvest. Post-harvest contamination can occur if the drying is delayed and during storage of the crop if moisture is allowed to exceed critical values for mold growth. Delayed harvest in rainy weather frequently leads to grain's sprouting on the panicle, particularly for nondormant japonica rice. The fungi, *A. flavus*, *A. parasiticus*, *A. niger*, and *A. ochraceus*, have been reported earlier by Reddy and colleagues (2006), of which *A. flavus* have been identified as the primary quality deterrent, producing aflatoxin-contaminated seeds when in storage (Desai and Ghosh, 2003; Reddy et al., 2005).Reddy (2008) explored the incidence of *Aspergillus* sp. in 1,200 rice samples consisting of paddy (675) and milled rice (525) collected from 43 locations in 20 rice-growing states across India. The seeds collected were either from areas exposed to different weather conditions or stored at various storage conditions, namely seeds from the crop exposed to heavy rains and floods,

seeds from submerged or damp conditions, seeds stored in the warehouse for 1 to 4 years, or seeds from the grain market. *A. flavus* and *A. niger* dominated in almost all the seed samples (Tables 1 and 2).The incidence of heavy rains during the harvesting season in India favors aflatoxin contamination of the rice crop (Tulpule et al., 1982). Fungal infection was more frequent in parboiled dried paddy and milled parboiled rice. Of the various stages, rice at the drying stage and the stage preceding milling were shown to contain aflatoxins (Kumar et al., 2008). *Aspergillus* species are common contaminants in stored rice and their incidence increases with the infestation of rice weevil (*Sitophilus oryzae*) (Prasad et al., 1987; Choudhury et al., 1999). In addition to rice, Reddy and colleagues (2005),Reddy and Raghavender (2006) and Raghavender and colleagues (2007) reported that *A. flavus* incidence increases with the infestation of insects in horsegram, sorghum, and pearl millet, respectively.

Udagawa (1976) isolated *A. candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. versicolor*, *Chaetomium globosum,* and *P. citrinum* from milled rice from Malaysia. Fouzia and Samajpati (2000) isolated mycotoxin-producing fungi from contaminated grains of rice sold in the local markets of Calcutta, India. It was found that AFB1 was produced by *A. flavus* and *A. parasiticus*, aflatoxin G_1 by *A. flavus*, ochratoxin by *A. ochraceus*, sterigmatocystin by *A. japonicus*, and citrinin by *P. citrinum*.

Waghray and colleagues (1988) reported *Aspergillus* sp. as the most predominant fungi in the grain samples of flood-affected paddy variety NLR 9672 collected from standing crop, threshing floors, and storage sites in the Nellore district of Andhra Pradesh. When the rice crop was exposed to frequent, heavy rainfall and floods, it was vulnerable to infection by *Aspergillus* sp. (Reddy et al., 2004). In an assay of 150 samples of stored rice grains from three different storage houses from Tamil Nadu, *Aspergillus* sp. accounted for >40% of the total count, followed by *Penicillium* sp. (24%), *Fusarium* sp. (13%), and other genera (23%) (Sundaram et al., 1988). Surveys in several parts of Tamil Nadu showed that fungal contamination of paddy (with husk) was greater than that on rice (without husk) and consisted mainly of field fungi. (Palaniswami et al., 1989). Almeida and colleagues (1991) collected 90 samples of milled rice from different regions of Brazil and evaluated them for seed mycoflora and aflatoxigenic strains

TABLE 1 Paddy Samples Collected, Samples Positive to *Aspergillus* spp. Infection and Number of Colonies Detected

States	Samples (No.)	Samples positive (No.)				Total colonies (No.)*			
		Af	An	Ao	Ap	Af	An	Ao	Ap
Andhra Pradesh	215	182	181	11	56	4584	3880	39	341
Andaman & Nicobar	34	33	33	5	19	1552	1207	7	166
Assam	40	27	32	5	21	996	347	187	283
Bihar	24	16	10	11	10	656	41	719	41
Chattishgarh	11	10	11	1	1	171	504	1	8
Gujarat	12	7	8	1	4	32	30	13	29
Jammu & Kashmir	27	27	11	0	0	348	28	0	0
Jarkhand	21	21	21	0	10	495	586	0	36
Karnataka	63	54	62	4	10	839	1015	6	15
Kerala	14	14	5	10	3	182	13	51	5
Maharashtra	7	6	7	0	2	149	166	0	2
Madhya Pradesh	19	19	19	1	3	396	374	7	19
Megalaya	9	4	3	5	7	20	97	10	20
Puducherry	38	37	36	3	14	664	941	4	43
Rajasthan	10	10	10	9	8	270	145	36	72
Tamil Nadu	42	42	37	11	17	477	241	5	106
Tripura	11	11	9	5	8	663	134	145	181
Uttaranchal	28	24	28	0	14	258	489	0	50
West Bengal	50	47	27	3	10	1948	621	286	290
Total	675	591	550	85	217	14700	10859	1516	1707

Af = Aspergillus flavus, An = Aspergillus niger; Ao = Aspergillus ochraceus, Ap = Aspergillus parasiticus.
*Total colonies = colonies emerged from rice grain samples plated on rose bengal agar.

TABLE 2 Rice Samples Collected, Samples Positive to *Aspergillus* spp. Infection and Number of Colonies Detected

States	Samples (No.)	Samples positive (No.)				Total colonies (No.)*			
		Af	An	Ao	Ap	Af	An	Ao	Ap
Andhra Pradesh	42	23	35	2	9	701	371	2	27
Delhi	122	117	105	10	11	2269	1225	49	27
Karnataka	29	24	28	2	7	226	369	2	10
Kerala	280	187	146	50	38	3469	3778	400	331
Puducherry	9	9	9	0	3	264	153	0	25
Tamil Nadu	43	39	38	3	39	829	515	3	28
Total	525	399	361	67	107	7758	6411	456	448

Af = Aspergillus flavus, An = Aspergillus niger, Ao = Aspergillus ochraceus, Ap = Aspergillus parasiticus.
*Total colonies = colonies emerged from rice grain samples plated on rose bengal agar.

of *Aspergillus*. They reported *Aspergillus*, *Penicillium*, *Cladosporium*, *Rhizopus*, and *Rhodotorula* species along with nonsporulating fungi. Among the *Aspergilli*, *A. parasiticus* was the most frequent species isolated from samples with moisture contents of 14% to 17%. *A. flavus* was the second most frequent species, isolated in samples with moisture contents of 14% to 16%.

Jayaraman and Kalyanasundaram (1990) collected 34 samples of rice bran and reported *A. flavus* as the major contaminant. Chary and Reddy (1987) reported 14 fungal species representing six genera that were isolated from rice. The predominant isolates were *Rhizopus stolonifer*, *P. citrinum*, and *A. flavus*. Toxigenic isolates included *A. flavus* (producing aflatoxin), *A. fumigatus* (gliotoxin), *A. nidulans* (sterigmatocystin), *Fusarium oxysporum* (fusarenon-X), and *P. citrinum* (citrinin). Abbas and colleagues (1997, 1999) reported the incidence of *F. proliferatum* in unpolished rice. Trung and colleagues (2001) had reported 43.8% *Aspergillus* sp., 21.9% *Fusarium* sp., 10.9% *Penicllium* sp., and 23.4% of other fungi, which were encountered in 25 samples of Vietnamese rice from the Mekong delta. Makun and colleagues (2007) found *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Mucor*, *Rhizopus*, *Trichoderma*, *Curvularia*, *Helmenthosporium*, and *Cladosporia* in 196 moldy rice samples in Nigeria.

Site of Infection of Mycotoxigenic Fungi in Rice Grains

Rice stored in warehouses and mills is known to harbor toxigenic fungal species. Many cultivars are grown in different rice ecosystems. *Aspergilli* are the major contaminants in rice seeds produced and stored under high humidity conditions. Reddy and colleagues (2006) studied the level of fungal contamination at different sites of seeds that were stored for more than a year using samples of 18 cultivars collected across rice ecosystems in India. In seed component plating, *Aspergilli* dominated as externally borne contaminants in unsterilized seed, kernel, hull, and kernel powder. After surface sterilization, only a limited number of colonies were obtained from seed, hull, kernel, and kernel powder in tests with seeds. Paddy seeds in Thailand contained high levels of a wide variety of fungi, while milled rice contained very few fungal contaminants (Pitt et al., 1994).

A high incidence of *A. flavus* was found in the seed mycoflora of rice bran (Elangovan et al., 1999). Desjardins and colleagues (2000) isolated 11 *Fusarium* sp. from paddy seed samples from fields at the foothills of the Himalayas in Nepal. Rough rice samples from warehouses in Brazil were milled to separate polished rice, bran, and rice hull to evaluate mycoflora in each of the samples (Carlos et al., 2000). The most frequent contaminants in those samples belonged to the genus *Aspergillus*. Taligoola and colleagues (2004) investigated fungal contaminants in locally milled rice grains and grains imported into Kampala in Uganda. They found over 60 species belonging to 34 genera in local and imported grains. The major contaminants were *A. candidus*, *A. flavus*, *A. niger*, *Eurotium amstelodami*, *E. rubrum*, *P. citrinum*, and *Talaromyces* sp. Sales and Yoshizawa (2005a, 2005b) described the incidence of *A. flavus* and *A. parasiticus* in rice bran (14%) and rough rice (78%).

Hafez and colleagues (2004) reported 120 species belonging to 38 genera in 64 paddy samples from Egypt. They found *A. flavus*, *A. sydowi*, *A. terreus*, *A. fumigatus*, *A. ochraceus*, *P. chryso-genum*, *P. corylophilum*, *F. oxysporum*, *Alternaria tenuis*, *Cladosporium cladosporioides*, *Trichoderma viride*, and *Mucor racemosus* more frequently on glucose agar. Despite such studies, there has been no attempt to investigate fungal contamination in totality in seeds, hull, rice kernel, and kernel powder of rice cultivars grown across the country in different ecosystems. Reddy and colleagues (2006) separated seed parts under aseptic conditions and estimated the level of contamination with *Aspergilli*. The results demonstrated that the seeds of cultivars show significant differences in the contaminating species and in their infection counts within seeds. Only fungal species, namely *A. flavus*, *A. niger*, *A. parasiticus*, and *A. ochraceus*, dominated as contaminants in different parts of the rice seeds. *Aspergilli* that were capable of producing AFB1 were found in all the rice cultivars at one site or another in seeds. The detection of fungal colonies in all the samples obtained after surface sterilizing in seed, hull, separated kernel, and kernel powder indicated that the infection from *A. flavus* and *A. niger*, as indicated by agar plate tests, and *A. flavus* and *A. parasiticus*, as indicated by serial dilutions tests, reached the starch in rice grains. In seed samples stored for 12 to 24 months, 60 to 130×10^3 cfu/g seed were detected. The higher levels of *Aspergilli* contaminations

detected on seeds of Manoharsali (31×10^4 cfu/g) and Cotton-dora sannalu (63×10^4 cfu/g) might be due to improper storage of seed, especially under high humidity conditions in the tropics. Although *Aspergillus* species were detected, aflatoxin contamination in rice was reported to be extremely low (Palaniswami et al., 1989). In a study on the environmental mycoflora of rice mills, Desai and Ghosh (2003) found the dominance of *Aspergillus* sp. They demonstrated that rice mill workers are occupationally exposed to airborne aflatoxin-producing strains. Reddy and colleagues (2006) showed the external and internal growth of *Aspergilli* in stored rice grains that were milled after a period of storage. Heavy contamination of *Aspergilli* in seeds of all cultivars kept in warehouses apparently caused respiratory problems to rice mill workers. Further investigations on the levels of colonization of the *Aspergilli* and aflatoxin, particularly in milled grains that are sold in market, may help to determine the level of health hazard from an exposure to such contaminated rice or on consuming them. It is also important to improve storage structures and conditions to ensure that these toxigenic fungi are not provided with suitable conditions for growth and production of toxins in rice grains.

Colonization of Mycotoxigenic Fungi on Rice Grains

Discolored grains are frequently encountered in marketed rice and they reduce quality and price. To detect the extent of fungal invasion of kernel starch, discolored kernels were inspected by scanning electron microscopy (SEM) (Mangala et al., 2006). These experiments showed the presence of *Aspergillus* sp. in kernels of rice cultivars that had invaded starch, endosperm. and embryo (Figure 1). This is the first report on the fungal invasion of *Aspergillus* sp. in rice kernel as studied directly using SEM. Mycock and colleagues (1990) studied *A. flavus* var. *columnaris* causing maize seedling infection using SEM. They showed that the fungus was able to invade the internal tissues and observed the hyphae in the xylem of the peduncle of maize seed. Similarly Klich and colleagues (1986) reported the presence of hyphae of *Aspergillus* sp. in the xylem of the outer pigment layer of cotton-seed. Balajee and colleagues (2005) made phenotypic analyses of *A. fumigatus* on different media using differential interference
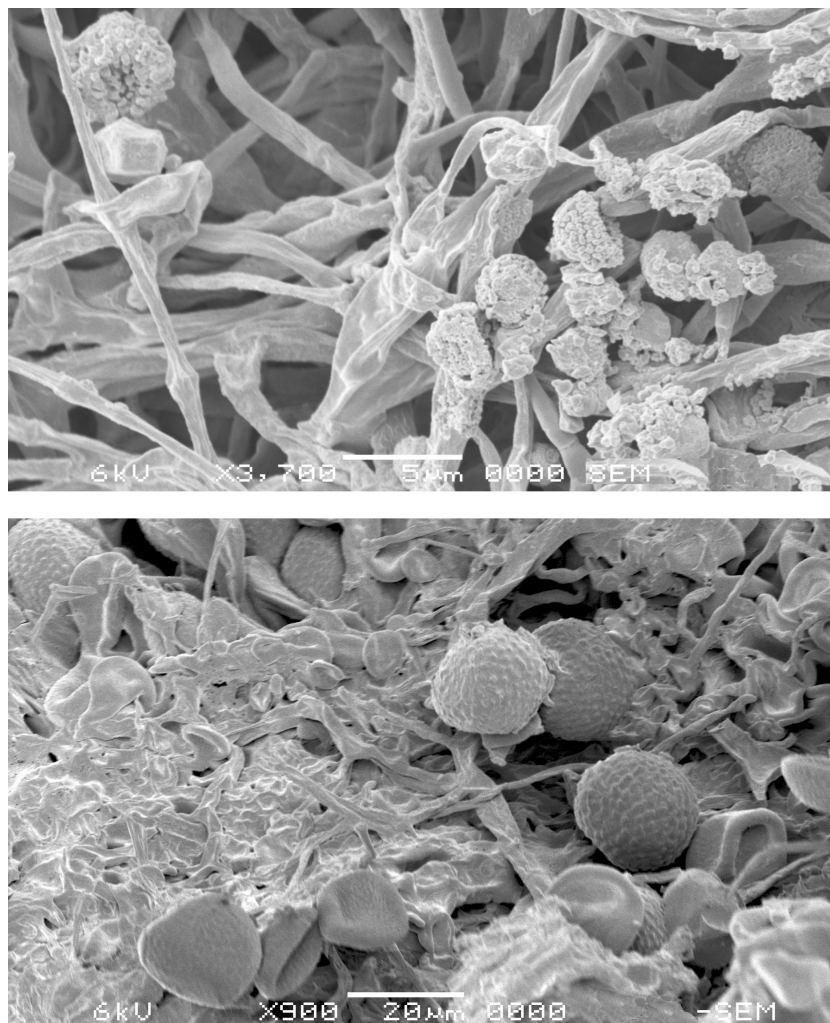
FIGURE 1 SEM showing hyphae with stipe, vesicles, and primorida of philalides (top); SEM showing hyphae and globose to subglobose structures, probably young vesicles with primordia of phialides (bottom) on rice grains.

contrast microscopy and SEM images. Ma and colleagues (2005) studied the surface ultrastructure and elasticity in growing tips and mature regions of *Aspergillus* hyphae under atomic force microscope (AFM) and cryo-SEM. Most of these studies on cereal grain infection by *Aspergilli* were either made by artificial inoculations or by using in vitro cultures.

Singotamu and Shetty (1998) examined the black colored rice kernel under SEM and observed bunches of spherical spores within the cellular boundaries of the kernel in between the medulla and cortical regions. They suspected germinating spores with germ tubes due to the presence of the *Fusarium* sp. or *Aspergillus* sp. The identity of the fungal contaminant was not clearly discernable. SEM studies had indicated that *Alternaria* sp. penetrated seed coat either by direct penetration through cuticle or through micropyle (Vaughan et al., 1988). SEM also revealed the direct penetration by *F. moniliforme* hyphae through epidermal cells of the seedlings and colonization of the host tissue by inter- and intracellular modes of growth (Murillo et al., 1999).

It is clear from our SEM studies that *Aspergilli* entered through crevices and cracks in starch grain. These cracks and fractures in the starch granula were much larger in size and were apparently caused by the growth and invasion of *Aspergilli*. Further, such infection apparently led to production of enzymes that digest starch grains and create wide crevices. The internal grain starch milieu appears to encourage invasive species of *Aspergillus* to extensively colonize rice kernels (Mangala et al., 2006). Mycock and Berjak (1991) examined four species of *Aspergillus* under SEM and found that a minimal distortion or collapse can be achieved with concomitant preservation of surface morphology. Chulim and colleagues (2003) used SEM to determine the morphology and degree of degradation in starch and acrylic-grafted starch by *A. niger*. Smith and Lineback (1975) examined the action of glucoamylase I and II from *A. niger* and the glucoamylase from *Rhizopus niveus* on native wheat and corn starch granules under SEM and observed some disc-like depression that produced small grooves on the surface of the granule.

Mycotoxins in Rice Grains

In India, the level of mycotoxins in rice differs from one location to another. This is due to various factors like temperature, relative humidity, and agricultural practices. In general, hot and humid conditions are very favorable for the growth of toxigenic fungi and mycotoxin production in agricultural produce. Many countries regulate specific mycotoxins and most countries try to limit exposure to the toxins. There are several systematic surveys on

mycotoxin levels in different agricultural commodities including
rice.

Aflatoxin Levels in Rice Grains

Reddy (2008) surveyed a total of 1,200 rice samples consisting of
paddy (675) and rice grains (525) and estimated AFB1 by indirect
competitive Enzyme Linked Imunosorbent Assay (ELISA). Of
this, 67.8% of the samples were AFB1-positive, ranging from 0.5
to 38.5 μg kg^{-1}. The highest AFB1 concentration was found in
samples collected from Maharashtra (38.5 μg kg^{-1}). The highest
aflatoxin concentration was also found in open samples exposed
to rain and submerged/damp conditions (12.9 μg kg^{-1}) that were
collected from different rice-growing areas of India. (Table 3).

TABLE 3 Paddy Samples Collected and Levels of AFB1 in Different States

States	Samples	Positive Samples (No.)	Positive (%)	Afla B$_1$-average (μgkg^{-1})
Andhra Pradesh	215	132	61.4	4.4
Andaman & Nicobar	34	27	79.4	3.1
Assam	40	33	82.5	7.0
Bihar	24	17	70.8	1.5
Chattishgarh	11	11	100.0	2.6
Gujarat	12	11	91.7	1.9
Jarkhand	21	16	76.2	1.6
Jammu & Kashmir	27	20	74.1	1.8
Karnataka	63	47	74.6	9.8
Kerala	14	7	50.0	1.4
Madhya Pradesh	19	16	84.2	8.5
Maharashtra	7	6	85.7	38.5
Megalaya	9	9	100.0	7.4
Puducherry	38	17	44.7	0.7
Rajasthan	10	2	20.0	0.9
Tamil Nadu	42	42	100.0	17.6
Tripura	11	9	81.8	6.3
Uttaranchal	28	19	67.9	1.1
West Bengal	50	36	72.0	2.0
Total	675	477	70.7	-

Afla B$_1$ = aflatoxin B$_1$.

Liu and colleagues (2006) reported all four aflatoxin (AFB1, AFB2, AFG1, and AFG2) levels in 36 dehusked brown rice samples in China ranging from 0.99 to 3.87 μg kg^{-1}. Bhat and Krishnamachari (1978) reported a high amount of aflatoxin in a wide variety of food, namely parboiled rice, maize, groundnut, and so on. Pande and colleagues (1990) reported that the quantity of aflatoxin was higher in rice samples compared with wheat and maize. Prasad and colleagues (1987) tested 56 samples of stored rice and 12 were positive for aflatoxin. Levels of aflatoxins ranged from 184 to 2830 μg kg^{-1}. Studies by Prasad and colleagues (1986) and Pawan and colleagues (1990) showed that a number of samples of stored paddy rice were contaminated with AFB1, AFG$_1$, and AFG$_2$. Higher moisture content of the grains at the time of storage increased the *Aspergillus* infection and aflatoxin contamination (Nandi and Haggblom, 1984). Jayaraman and Kalyansundaram (1990) reported that 35% of the samples of raw rice bran and parboiled rice bran showed the presence of AFB1. However, it was shown that bran of parboiled rice supported higher aflatoxin production than bran of raw rice (Jayaraman and Kalyansundaram, 1994).

In a study from Sri Lanka, Bandara and colleagues (1991) reported that in almost all the samples of parboiled rice, AFB1 and AFG1 contents were significantly higher than in raw milled rice. Cultivar differences in the amount of AFB1 and AFG$_1$ were shown by Sinha and Dubey (1991). Kim and Lee (1996), using ELISA and immunohistochemical staining, revealed that the edible portion of inoculated grains exhibited significantly higher levels of toxin than did the rice hulls, and the embryo contained a higher proportion of toxins than the endosperm. In another study with rice bran samples taken from rice mills in coastal and interior districts of Andhra Pradesh, Karnataka, and Tamil Nadu, nearly 62% of samples showed AFB1 at low levels. One-third was in the 50 to 500 μg kg^{-1} range, while another third of the samples had up to 2000 μg kg^{-1} (Elangovan et al., 1999). Poor infrastructure, hygiene, and sanitary conditions at the mills were identified as the reasons for AFB1 contamination in rice bran samples, which are more prone to invasion by the *Aspergillus* sp.

In seed samples from the threshing floor, where *A. flavus* was detected after storage for 1 year, AFB1 was estimated at 32 μg kg^{-1}. In the dehusked seed samples, the concentration

of AFB1 increased from 30 μg kg^{-1} prior to storage to 60 μg kg^{-1} after storage (Waghray et al., 1988). A survey conducted by Food Standards Agency, United Kingdom, in 2002 to determine the levels of mycotoxins present in a range of rice (long grain rice, easy cook rice, basmati rice, specialty rice, brown rice, short grain rice, flaked rice, and ground rice) available at retail outlets in the United Kingdom indicated the absence of ochratoxin A, sterigmatocystin, fumonisin B_1, B_2, and B_3, and zearalenone. Aflatoxins in the rice samples analyzed were at levels ranging from 0.2 to 1.8 μg kg^{-1}. All levels found were below the European Commission legislative limits of 2 μg kg^{-1} AFB1 and 4 μg kg^{-1} total aflatoxin in cereal products for direct human consumption. A low level of DON (12 μg kg^{-1}) was detected in one sample of rice.

Palaniswami and colleagues (1989) reported that AFB1 levels ranged from a trace to 40 μg kg^{-1} in rice and up to 20 μg kg^{-1} in paddy. Rice and paddy supplied through the public distribution system did not show AFB1 above tolerance levels. In almost all the samples of parboiled rice examined, the AFB1 and AFG1 contents were significantly higher than in raw milled rice. The highest AFB1 content was 185 μg kg^{-1} and the highest AFG1 content 963 μg kg^{-1}. The total aflatoxin levels in eight samples of polished and brown rice were determined by an immunoaffinity column clean-up method coupled with High Performance Liquid Chromatography (HPLC). They reported that the incidence of aflatoxin in polished and brown rice was 94% and 100%, respectively (Sales and Yoshijawa, 2005a). Fifteen hundred eleven (1,511) samples of parboiled rice were collected from rural and urban areas of 11 states in India and analyzed for AFB1 contamination. Of this, 38.5% of the parboiled rice samples were contaminated with AFB1 at 5 μg kg^{-1} and 17% of the samples showed the presence of AFB1 above the Indian regulatory limit of 30 μg kg^{-1} (Toteja et al., 2006).

A survey on aflatoxin contamination in stored seeds of eight paddy varieties commonly cultivated in three districts was conducted. Among 82 fluorescence-positive samples (from a total of 343 samples collected), 20 were contaminated with aflatoxins. AFB1 was found in 11 samples; AFB_1 and AFB_2 in 5 samples; AFB_1, AFG_1, and AFG_2 in 2 samples; and AFB_1 and AFG_2 in 2 samples (Pawan et al., 1990). Thirty-four samples of rice bran, 9 of which were from raw (untreated) rice and 25 from parboiled

TABLE 4 Rice Samples Collected and Levels of AFB1 in Different States

States	Samples (No.)	Positive Samples (No.)	Positive (%)	AflaB$_1$-average (μgkg^{-1})
Andhra Pradesh	42	20	47.6	2.3
Delhi	122	96	78.7	3.5
Karnataka	29	12	41.4	0.7
Kerala	280	183	65.3	1.5
Puducherry	9	2	22.2	0.5
Tamil Nadu	43	24	55.8	2.8
Total	525	337	64.1	—

Afla B$_1$ = aflatoxin B$_1$.

rice, were collected from commercial rice mills in and around Madras and analyzed for mycotoxins. Five of the 9 raw rice samples and six of the 25 parboiled rice samples were positive for aflatoxins (Jayaraman and Kalyanasundaram, 1990). Aflatoxin content was measured in 67 composite rice samples prepared from 170 samples obtained from various storage systems in Bihar. Levels of aflatoxin contamination for rice ranged from zero to 810 μg kg^{-1} (Jeswal, 1986). Reddy (2008) explored 525 milled rice samples collected from different states in the country, and 64.1% samples were positive for aflatoxin. AFB1 was recorded in samples of market yards from New Delhi (3.5 μg kg^{-1}), Tamil Nadu (2.8 μg kg^{-1}), Andhra Pradesh (2.3 μg kg^{-1}), and Kerala (1.5 μg kg^{-1}) (Table 4).

A total of 581 samples, comprising 197 of maize and maize products, 197 of rice, 5 of wild black rice, 31 of millet, 33 of buckwheat, and 118 of pulses were analyzed for aflatoxins B$_1$, B$_2$, G$_1$, and G$_2$ by HPLC. Wild rice had a relatively high incidence of aflatoxins, with two of five samples containing >1 μg kg^{-1}. Most other cereals and pulses studied had low incidence and concentration of aflatoxins (Madsen and Rasmussen, 1990). Thirty samples each of wheat and rice and 22 of maize were screened qualitatively and quantitatively for the presence of mycotoxins, out of which 13, 15, and 7 samples, respectively, were found to be contaminated with different mycotoxins. Aflatoxin was present in 37.4% of the samples and ochratoxin A, sterigmatocystin, citrinin, rubratoxin, and zearalenone were also present in the cereals. Out of 597 samples

of rice tested, AFB1 was not detected in 525 (minimum detectable level 12 μg kg^{-1}). AFB1 was present at 30 μgkg^{-1} in 12 and at 12 to 30 μg kg^{-1} in 57 samples of parboiled rice (Breckenridge et al., 1986). In a survey of mycotoxins in commonly consumed cereal foodstuffs in 1981, 74 of 137 samples from houses and markets contained mycotoxins ranging in concentration from 8 to 750 μg kg^{-1}. The maximum percentage contamination was recorded in maize (63.33%), followed by sorghum (60%), broken rice (56.25%), ragi (40.54%), and parboiled rice (33.33%) (Reddy et al., 1986).

Other Mycotoxins in Rice Grains

Very few scattered reports on other mycotoxins, namely fumonisins, ochratoxin, zearalenone, dexoynivalenol, and sterigmatocystin in rice grains, have been reported. Abbas and colleagues (1999) previously reported the incidence of fumonisin B_1, moniliformin, zearalenone, and DON. In another study, Abbas and colleagues (1997) detected fumonisin B_1, B_2, and B_3 in 20 samples of rough rice collected during the 1995 harvest season from Arkansas and Texas. Makun and colleagues (2007) determined ochratoxin A in 56 samples with concentrations between 24 and 1,164 μgkg^{-1} and zearalenone ranging between 24 and 1,169 μgkg^{-1} in 93 samples collected from Nigeria. Trung and colleagues (2001) detected ochratoxin A in rice samples from south Vietnam. In another systematic study, Tanaka and colleagues (2007) reported the incidence of fumonisins, DON, zearalenone, sterigmatocystin, ochratoxin, nivalenol, and citrinin in rice. Konishi and colleagues (2006) reported the natural contamination of ochratoxin A in retailed rice in 2004 and 2005 in Japan. Reddy, Reddy, and Muralidharan (2007) and Pena and colleagues (2005) have reported the presence of ochratoxin A in rice samples from India and Portugal, respectively.

Management of Mycotoxigenic Fungi Using Botanicals, Microbiologicals and Cooking Methods

Botanicals

Antifungal chemicals have been used for the preservation of stored grains (Paster et al., 1995). Health hazards from exposure

to toxic chemicals and economic considerations make natural plant extracts ideal alternatives to protect food and feed from fungal contamination (Chin and Cheng, 1998). Clove has been shown to possess antimicrobial activity against three potent food-borne pathogens, namely *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus cereus*, which are responsible for many health-related problems (Sofia et al., 2007). In our investigation, clove effectively inhibited the mycelial growth of *A. flavus* and aflatoxin production (Reddy et al., 2008).

Clove is an extremely safe and consumer-beneficial treatment alternative to prevent storage fungi in rice grains. Use of clove and purified eugenol standards in dentistry is well known (Miller et al., 1979). Eugenol has been extracted and purified from cloves (Hitokoto et al., 1980) and from *Ocimum gratissimum* (Faria et al., 2006). Several reports are available on the inhibitory effect of clove on *A. flavus* and *A. parasiticus* as well as several other fungi (Bullerman et al., 1977; Mabrouk and Shayeb, 1980). However, such studies have not been made on *Aspergilli* contaminating rice grains during storage. Therefore, eugenol extracted from clove was characterized and tested for its antifungal activity against *Aspergillus* sp. that contaminated and discolored rice in storage. This purified eugenol from clove extracts showed clear inhibition of the mycelial growth of *Aspergillus* sp. at 4.8 mg/disc and complete inhibition of mycelial growth of *A. flavus* on Thin Layer Chromatography (TLC) bioassay (Figure 2). On rice treated at 2.4 mg eugenol/g of grains, the inoculum of *A. flavus* failed to grow and thus AFB1 biosynthesis on rice was prevented (Figure 2) (Reddy, Reddy, Prameela et al., 2007). Hitokoto and colleagues (1980) reported the extraction of eugenol from clove and identified it on TLC with an R_f of 0.5. Faria and colleagues (2006) identified eugenol from extracts of *O. gratissimum* on TLC with an R_f of 0.43 and carried out its purification by column chromatography. But in these two studies, the purity of eugenol was not reported. The purity of eugenol (79.3%) extracted from cloves as estimated by HPLC (Reddy, Reddy, Prameela et al., 2007) (Figure 3). Saito and colleagues (2004) reported similar results of spectral analysis in eugenol samples. Faria and colleagues (2006) reported that the molecular weight of eugenol purified from *O. gratissimum* was 164, very similar to M/z 165 (m+1 peak) estimated for eugenol from clove (Reddy, Reddy, Prameela et al., 2007) (Figure 4).
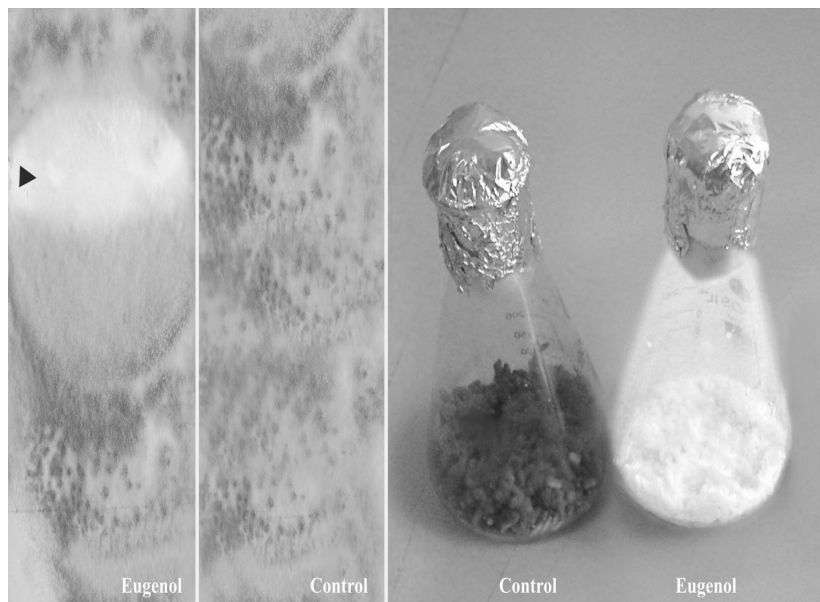
FIGURE 2 Eugenol spot at Rf 0.5 on TLC plate completely inhibited mycelial growth of *A. flavus* and control with full mycelial growth; absence of fungal growth in *A. flavus* inoculated flasks, control and eugenol treated (2.4 mg/g).

Jham and colleagues (2005) reported antifungal activity of cinnamon bark oil against *A. flavus* and *A. niger*. Juglal and colleagues (2002) studied the effectiveness of nine essential oils to control the growth of mycotoxin-producing molds and noted that clove, cinnamon, and oregano were able to prevent the growth of *A. parasiticus* and *F. moniliforme*, while clove (ground and essential oil) markedly reduced the aflatoxin synthesis in infected grains. Although clove extracts from a few plant sources were investigated, bioassay was neglected. More than 280 plant species have been investigated for their inhibitory effect on toxigenic *Aspergilli* and nearly 100 of these plants had some activity on growth or toxin production by fungi (Belmont and Carvajal, 1998).

Hitokoto and colleagues (1980) reported the complete inhibition of mycelial growth of *A. flavus* and *A. versicolor* at 250 μg eugenol/ml of yeast-sucrose broth, but the biosynthesis of AFB1 in vitro was arrested only at 125 μgml^{-1} of eugenol. Karapýnar (1989) reported the inhibitory effect of crude extracts from

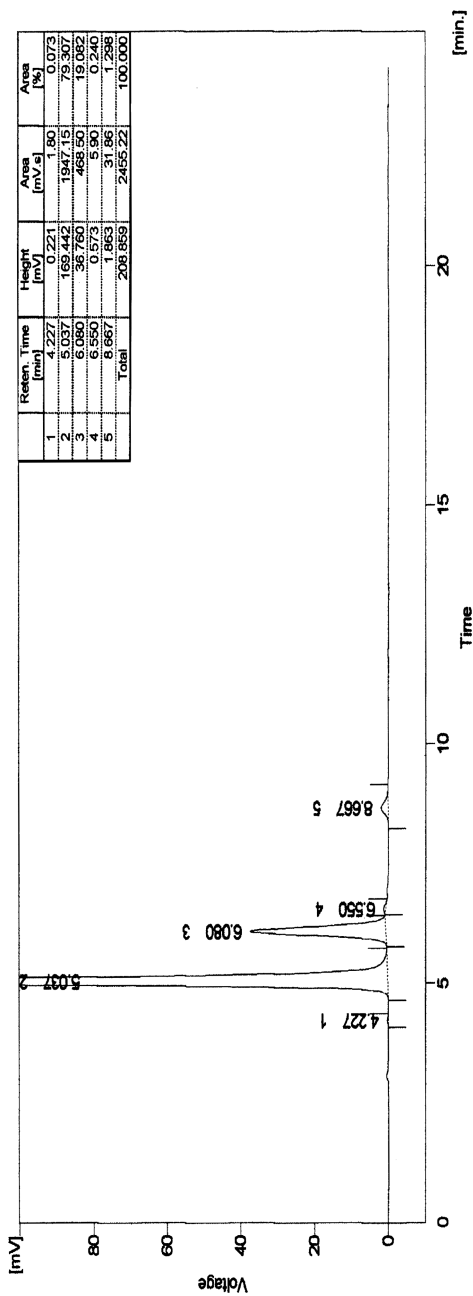|	Reten. Time [min]	Height [mV]	Area [mV.s]	Area [%]
1	4.227	0.221	1.80	0.073
2	5.037	169.442	1947.15	79.307
3	6.080	36.760	468.50	19.082
4	6.550	0.573	5.90	0.240
5	8.667	1.863	31.86	1.298
Total		208.859	2455.22	100.000

FIGURE 3 HPLC chromatogram of eugenol extracted from clove. 5.037 peak confers eugenol in the above chromatogram.
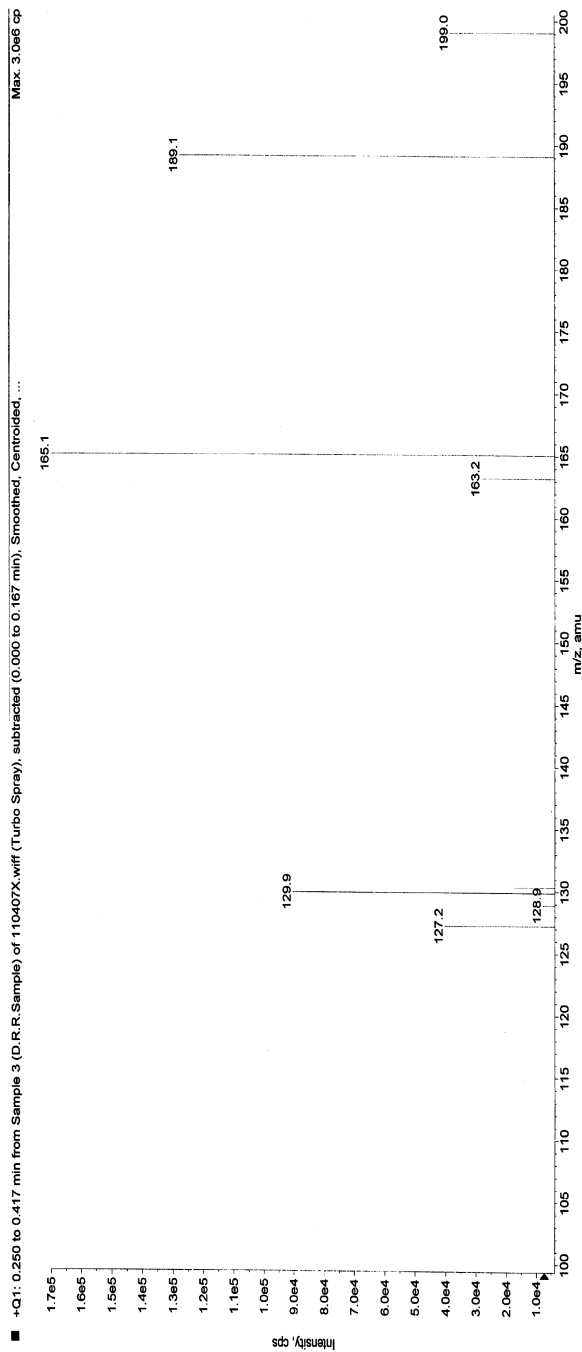
305

FIGURE 4 Mass spectrum of eugenol extracted from clove.

mint, sage, bay, anise, and ground red pepper (0.5%, 1.0%, 2.0%, 4.0%, 8.0%, 16.0% w/v) on the growth of *A. parasiticus* NRRL 2999 and its aflatoxin production in vitro. Akgul and Kivanç (1988) studied antifungal activity of selected Turkish spices (black cumin, coriander, cumin, dill, laurel, oregano, parsley, spearmint, white mustard) on some food-borne fungi and found that ground oregano (1.0%, 1.5%, 2.0% w/v) and its essential oil (0.05%, 0.025%) showed an inhibitory effect on *A. flavus, A. niger*, *Geotrichum candidum*, *Mucor* sp., and *P. roqueforti*. They found oregano essential oil to exhibit a higher inhibitory effect than sorbic acid. Saxena and Mathela (1996) found antifungal activity of new compounds from *Nepeta leucophylla* and *N. clarkei* against *Aspergillus* and *Penicillium* sp. Reddy, Reddy, and Muralidharan (2007) reported the efficacy of certain plant extracts on mycelial growth of *A. ochraceus*.

Mathela (1981) screened 12 terpenoids against growth of *Aspergillus*, *Penicillium*, and *Fusarium* species and found thymol and carvacrol to be more active than nystatin and talsutin. Clove completely inhibited the mycelial growth of *A. flavus* and afla-toxin formation at 0.1% (Mabrouk and Shayeb, 1980). The effects of cinnamon oil, clove oil, cinnamic aldehyde, and eugenol on growth and aflatoxin production by *A. parasiticus* were studied earlier using YES media as the substrate. All four substances inhib-ited mold growth and subsequent toxin production (Bullerman et al., 1977). Most research work on the effects of clove, eugenol, and other plant-derived chemicals were made in vitro or with nontarget organisms in bioassay tests. We have demonstrated the ability of crude clove extracts and purified eugenol to inhibit *Aspergilli* and arrest colonization of rice grains. Eugenol is the major antifungal component of clove that effectively inhibited fungal growth of *A. flavus*, *A. parasiticus*, *A. niger*, and *A. ochraceus*. AFB1 biosynthesis was also inhibited, as there was no growth of fungus on eugenol-treated rice grains. Treatment with crude clove extract or purified eugenol can help prevent mycotoxin contamination in many foods (Reddy, Reddy, Prameela, et al., 2007).

Microbiologicals

The development of biological detoxification measures, especially in fermented food products, is essential to improve the safety of these foods for human consumption (Sweeney and Dobson, 1999). Of the *Trichoderma* isolates evaluated against mycelial growth of *Aspergilli* and AFB1 production by *A. flavus*, the culture filtrate of *Trichoderma virens* showed a complete inhibition of growth of *A. flavus* at 15% concentration and was significantly superior to all other *Trichoderma* sp. (Reddy et al., 2008). Calistru and colleagues (1997) also reported similar potential for biological control of *A. flavus* using *Trichoderma* sp., based on in vitro results. Lauzon and colleagues (1995) reported the inhibitory potential of *Cladosporium fulvum* against *A. parasiticus* in rice dextrose agar and in rice grains. Goldblatt (1971) reported that *Flavobacterium aurantiacum* used as inhibitory biocontrol agent against aflatoxin. Choudhary (1992) reported that *T. viride* was found to be effective in inhibiting the growth of *A. flavus* isolated from groundnut seeds to an extent of 72.5% in vitro. Desai and colleagues (2000) characterized 26 isolates of *Trichoderma* for biocontrol potential against *A. flavus* in dual culture technique and observed growth inhibition of *A. flavus* by production of nonvolatile antibiotics.

Studies on the effect of *Pseudomonas fluorescens* on mycelial growth and AFB1 production by *A. flavus* revealed that culture filtrates of DRPf 002 and DRPf 005 showed maximum growth inhibition (91%–98%) at 15% concentration (Reddy et al., 2008). Podile and Prakash (1996) found that *Bacillus subtilis* suppressed *A. niger* growth by 90% and bacterial cells multiplied in situ and colonized the mycelial surface. Narain and Mohanty (1983) reported the inhibition of growth of *A. flavus* and *A. niger* by *B. subtilis*. In our investigation the culture filtrate of *B. subtilis* effectively inhibited the mycelial growth of *A. flavus* ranging from 45% to 60% at 15% concentration and AFB1 (58%) at 200 mlkg^{-1} of rice (Reddy et al., 2008). *Bacillus pumilus* inhibited aflatoxin production and mycelial growth of *A. parasiticus* NRRL 2999 when both organisms were grown simultaneously in yeast extract sucrose broth. Percentages of inhibition of aflatoxin production ranged between 98.4% and 99.9% (Munimbazi and Bullerman, 1998).

Biological degradation of AFB1 by *Rhodococcus erythopolis* and *Mycobacterium fluoranthenivorans* was greater than 90% within 4 hours at 30°C, while after 8 hours AFB1 was practically not detectable. The degradation of AFB1 by these bacteria most probably occurred through a cascade of enzyme reactions with loss of fluorescence over time (Teniola et al., 2005). The biodegradation of AFB1 by *R. erythropolis* was examined in liquid cultures. The degradation was enzymatic and the enzymes responsible for the degradation of AFB1 were extracellular and constitutively produced (Alberts et al., 2006). In this investigation the culture filtrate of *R. erythopolis* completely inhibited the mycelial growth of *A. flavus* and AFB1 production at 25 mlkg^{-1} concentration (Reddy et al., 2008).

Decontamination of Mycotoxins in Rice by Cooking Methods

The use of heat treatment with the double aim of processing the food and reducing mycotoxin contamination has been investigated by several research groups. Samples of rice artificially contaminated with AFB1 and ochratoxin A were subjected to different types of cooking (Palavaras et al., 2004). The highest mycotoxin reductions were found when rice was cooked in excess water (86.7%–89.1%), by normal cooking (82.3%–83.1%) and finally by microwave oven (72.5%–82.4%). Reddy (2008) employed a wide array of physical decontamination strategies and effectively reduced the AFB1 levels in rice grains. He reported that cooking in a mud bowl on a gas stove reduced the AFB1 by 97.4% followed by cooking over firewood (76.3%), normal cooking (72%), and microwave oven cooking (52%) (Table 5). Castells and colleagues (2006) reported the reduction (51%–95%) of aflatoxins by extrusion cooking of rice meal. Another research group (Park and Kim, 2006) showed a high reduction (78%–88%) in AFB1 in rice by pressure cooking. The reduction of aflatoxin contamination in rice was also evaluated with normal cooking, cooking with excess water, and in a pressure cooker, the latter proving to be the most effective, showing a reduction of up to 75% of the initial contents (Rehana et al., 1979). Cazzaniga and colleagues (2001) reported on the reduction of DON in corn meal after extrusion cooking at two temperatures (150°C and 180°C). They concluded that temperature had a significant influence in reducing the

TABLE 5 Reduction of AFB1 in Rice by Different Cooking Methods

Type of cooking	Aflatoxin B_1 before cooking ($\mu g\,kg^{-1}$)	Aflatoxin B_1 after cooking ($\mu g\,kg^{-1}$)	Reduction (%)
Normal	61.3	17.6	72
Microwave oven	61.3	30.4	52
Firewood	38.5	9.3	76.3
Gas	38.5	1.2	97.4
CD (P = 0.05)	20.7	4.0	—
CV (%)	24.6	18.5	—

DON concentration but no differences were detected between the temperatures.

Conclusion

The experience of the past four decades indicates that it is possible to maintain present food consumption levels by increasing overall food supplies in quantitative terms. However, in terms of providing food of the right quality that is nutritious and free from mycotoxins, the task ahead is challenging, particularly in developing nations of the world. Many research institutes, including Directorate of Rice Research, India, have carried out research on mycotoxin contamination and developed technologies (viz. use of botanicals and microbiologicals) that can significantly reduce contamination, but these technologies are not adopted by the farmers due to lack of awareness. Hence this review aimed to document the level of knowledge and extent of adoption of mycotoxin management practices of rice and constraints faced by farmers in adoption of this technology through various programs. Finally, recent advances in mycotoxicology have made it possible to use this research for improving safe consumption of food that is free of mycotoxins.

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